In Situ Analyses of Methane Oxidation Associated with the Roots and Rhizomes of a Bur Reed, Sparganium eurycarpum, in a Maine Wetland.

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In Situ Analyses of Methane Oxidation Associated with the Roots and Rhizomes of a Bur Reed, *Sparganium eurycarpum*, in a Maine Wetland†

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Methane oxidation associated with the belowground tissues of a common aquatic macrophyte, the burweed *Sparganium eurycarpum*, was assayed in situ by a chamber technique with acetylene or methyl fluoride as a methanotrophic inhibitor at a headspace concentration of 3 to 4%. Acetylene and methyl fluoride inhibited both methane oxidation and peat methanogenesis. However, inhibition of methanogenesis resulted in no obvious short-term effect on methane fluxes. Since neither inhibitor adversely affected plant metabolism and both inhibited methanotrophy equally well, acetylene was employed for routine assays because of its low cost and ease of use. Root-associated methanotrophy consumed a variable but significant fraction of the total potential methane flux; values varied between 1 and 58% (mean ± standard deviation, 27.0% ± 6.0%) with no consistent temporal or spatial pattern during late summer. The absolute amount of methane oxidized was not correlated with the total potential methane flux; this suggested that parameters other than methane availability (e.g., oxygen availability) controlled the rates of methane oxidation. Estimates of diffusive methane flux and oxidation at the peat surface indicated that methane emission occurred primarily through aboveground plant tissues; the absolute magnitude of methane oxidation was also greater in association with roots than at the peat surface. However, the relative extent of oxidation was greater at the latter locus.

Wetlands currently account for a major percentage of atmospheric methane (10, 26) and have done so historically (2, 9). The significance of wetlands can be attributed in part to the relatively large fraction of primary production degraded under methanogenic conditions. In addition, aquatic plants promote methane ventilation to the atmosphere through their substantial belowground surface area and root and rhizome architecture (7, 12, 37, 38). Numerous studies have documented the extent of plant-mediated methane flux; values varied between 1 and 58% (mean ± standard deviation, 27.0% ± 6.0%) with no consistent temporal or spatial pattern during late summer. The absolute amount of methane oxidized was not correlated with the total potential methane flux; this suggested that parameters other than methane availability (e.g., oxygen availability) controlled the rates of methane oxidation. Estimates of diffusive methane flux and oxidation at the peat surface indicated that methane emission occurred primarily through aboveground plant tissues; the absolute magnitude of methane oxidation was also greater in association with roots than at the peat surface. However, the relative extent of oxidation was greater at the latter locus.

MATERIALS AND METHODS

Site description. Rates of methane emission and oxidation were assayed in a marsh located adjacent to Route 130, about 6.6 km south of Damariscotta, Maine. *Typha latifolia* dominates the wetland, but mixed to pure stands of a variety of other aquatic macrophytes occur, including *S. eurycarpum, P. cordata, S. lancifolia, and C. jamaicense*. The latter observations were consistent with results of King et al. (23), who showed that rates of in vitro methane consumption by *C. jamaicense* roots were relatively low. Much higher rates of methane oxidation by roots and rhizomes have been observed during in vitro assays of a diversity of other aquatic plants, including representatives of the three groups Sebacher et al. (38) identified on the basis of biomass-specific methane emission rates. Epp and Chanton (15) have also reported active methane consumption by intact specimens of *Pontederia cordata* and *Sagittaria lancifolia* during greenhouse incubations. However, Schipper and Reddy (36) suggested that greenhouse assays might overestimate methane oxidation.

This manuscript documents in situ assays of methane oxidation and emission from a stand of *Sparganium eurycarpum*. We used static chambers to measure emission and compared the efficacy of added acetylene or methyl fluoride as an inhibitor of methane oxidation. We also evaluated the effects of acetylene and methyl fluoride on photosynthesis and stomatal resistance in situ and in vitro and their effects on peat methanogenesis. The results suggested that root- and rhizome-associated methanotrophs consumed about 25% of the total methane flux (range, 1 to 58%) with no consistent temporal patterns between August and the occurrence of senescence in October. The extent of methane consumption was unaffected by water table fluctuations and was not correlated with ambient temperature. Acetylene and methyl fluoride were equally effective inhibitors of methane oxidation in situ, and neither affected photosynthesis or stomatal resistance. Although acetylene and methyl fluoride significantly inhibited peat methanogenesis in vitro, time course data suggested that any such effect was not manifested in decreased rates of methane emission in situ.

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relatively undecomposed peats in the marsh typically range from 1 to 2 m in depth. Standing water occurs from roughly October to June, with ice cover during winter; intermittent standing water typifies summer and early fall. Standing water occurs from roughly October to June, with ice cover during winter; intermittent standing water typifies summer and early fall. S. eurycarpum largely dominates the subtidal zone for this study, which was established adjacent to a small creek that bisects the marsh and is accessed via a boardwalk that was constructed to facilitate sampling and to minimize disturbance.

**Depth distribution of dissolved oxygen in marsh peat.** Oxygen profiles in the marsh peat were obtained using stirring-insensitive Clark-style microelectrodes with tip diameters of about 10 µm (34). Profiles were generated in situ by positioning the electrodes with a micromanipulator mounted on a tripod for stability. The electrode signals were detected with a Diamond Electrobotch oxygen sensor that also provided a polarizing voltage. Electrodes were standardized with air-saturated deionized water at ambient field temperature; the dissolved oxygen content of each of these solutions was estimated from oxygen solubility tables (25). Oxygen profiles were determined when the peat surface was illuminated by ambient sunlight (measured with the quantum sensor of a Li-Cor 6250) and after the water table levels were measured at several sites in the marsh by using wells constructed of 5-cm-inner-diameter polyvinyl chloride pipes inserted to a depth of about 1 m.

**Inhibition of peat methanogenesis and root methanotrophy by acetylene and methyl fluoride.** The effects of acetylene and methyl fluoride on peat methanogenesis were assessed by using 10-cm³ volumes of a slurry prepared by homogenizing the upper 10 cm of a peat core with 0.5 volume of pore water collected at the site; the slurry was prepared by anaerobic techniques (e.g., continuous flushing with nitrogen) to limit oxygen exposure. Slurries were dispensed into 120-cm³ serum bottles that were flushed with oxygen-free nitrogen and sealed with butyl rubber stoppers. Acetylene or methyl fluoride was added to the bottle headspaces at initial concentrations ranging from 0.01 to 1%; methane accumulation in the headspace was measured by periodically removing 0.3-cm³ subsamples for assay by gas chromatography (20, 21).

Approximately 0.5 g (fresh weight) of washed, sediment-free excised roots were incubated in stoppered 40-ml culture tubes (23) containing about 0.5% methane. Methane consumption was assayed by removing 0.3-cm³ subsamples for assay by gas chromatography (23). After establishing an initial rate of uptake, acetylene or methyl fluoride was added at 0.01, 0.1, or 1% (final concentration) to duplicate samples; a set of triplicate samples was maintained without inhibitor addition. The assay for methane consumption continued as described before.

**Assays for methane emission and oxidation by plants and peat.** Cylindrical static chambers (33-cm inner diameter by 63-cm height; 1-mm wall thickness) for flux assays were constructed of translucent greenhouse fiberglass that transmitted approximately 50% of the ambient light (400 to 700 nm) during field use as determined with the quantum sensor of a Li-Cor 6200 photosynthesis analyzer. The chambers enclosed four or fewer culms of S. eurycarpum and were deployed in an open configuration approximately 24 h prior to the initiation of flux assays; during this equilibration period, inner temperatures were equivalent to ambient values. Flux measurements were begun after the chambers were sealed with clear polyvinyl film; the film was stretched tightly over the top edge of the chamber (which had been coated with a thin layer of silicone rubber to promote a leak-free seal) and secured with elastic bands. Preliminary control assays showed that the chamber system lost negligible methane during periods longer than those used for typical incubations. For a given flux measurement, 10-cm³ headspace samples were removed with needles and syringes at 16- to 20-min intervals for 45 to 90 min. During these incubations, chamber temperatures rose to values ≤7°C above ambient temperature; methane accumulated linearly within the chambers during the incubations. Subsequent to the flux measurements, the polyvinyl film was removed and chambers were allowed to equilibrate with ambient conditions for at least several hours (typically >8 h) prior to the performance of any additional measurements.

Two or three measurements of rates of emission without methane oxidation inhibitors were obtained for each chamber over a period of about 24 h. These rates provided an estimate of net methane emission. A series of two to three flux measurements was also obtained after chambers were treated with acetylene or methyl fluoride. These rates provided a measure of maximum potential methane emission, with the difference between pre- and posttreatment rates being used as an estimate of methane oxidation; percent methane oxidation was calculated from the ratio of methane oxidation to maximum potential emission. To obtain maximum potential emission rates chambers were sealed as described above in the evening when shaded (about 1800 or later); a volume of methyl fluoride sufficient to produce a final headspace concentration of 3 to 4% was injected via syringe and needle into triplicate chambers. Alternatively, water was added to small beakers in triplicate chambers to hydrate an amount of calcium carbonate yielding a final headspace acetylene concentration of 3 to 4%. These concentrations were chosen on the basis of the results and recommendations of Epp and Chanton (15) for use of methyl fluoride. Chambers were incubated with inhibitors overnight (about 12 h minimum), opened for about 2 h to vent the headspaces, and then sealed for the assays as described above. A similar series of analyses was conducted with smaller chambers (6.3-cm inner diameter by about 20-cm height) that were deployed without enclosing aboveground plant biomass. Emission rates before and after addition of acetylene provided estimates of the flux of methane across the sediment surface relative to that through plants and the extent of oxidation at the sediment surface.

**Effect of acetylene and methyl fluoride on photosynthesis and stomatal conductance.** Rates of photosynthesis and stomatal conductance were assayed with a Li-Cor 6250 photosynthesis system fitted with a 250-cm² acrylic chamber (Li-Cor, Inc.). For in situ measurements, the midday photosynthetic activity of six control S. eurycarpum leaves from outside of the field chambers was compared to the activity of six replicate leaves from chambers treated with acetylene or methyl fluoride as described above. To facilitate this comparison, the chambers were removed prior to the assays. For in vitro analyses, S. eurycarpum was...
collected from the field and maintained separately with sediment in 10-liter plastic buckets. Chambers were created by sealing an inverted bucket over each plant container; acetylene or methyl fluoride was added at a final headspace concentration of 3 to 4%. Plants were incubated in darkness at ambient laboratory temperature for about 18 h. At the end of the incubation, the chamber tops were removed and the plants were held in ambient sunlight for 1 to 2 h. Photosynthetic activity and stomatal conductance were then measured for at least 5 leaves incubated with either acetylene or methyl fluoride and 10 leaves incubated in sealed chambers with no additions. Leaves of comparable size were chosen for each assay, and photosynthesis was measured with comparable illumination for all leaves. Photosynthesis rates were calculated from the means of three CO₂ uptake rates per leaf obtained for a small change in CO₂ partial pressure (with initial CO₂ partial pressure values close to ambient values) with small changes in relative humidity and incubation times typically <90 s.

Analysis of stem methane concentrations. Stem gases were collected from the leaves of *S. eurycarpum* and stems of *P. condensata* at intervals over a diurnal cycle. To minimize variability during sampling, a 28-gauge needle was inserted into the base of leaves of each plant and left in place; the needles were fitted with an adapter connected to a short length (15 cm) of Tygon tubing (0.8-mm inner diameter) terminated with a sampling port. This approach is based on a modification of methods of Dacey (11, 12). Gas samples (0.5 cm³) were obtained periodically for analysis of methane as described by King (21).

Plant biomass. Aboveground biomass was collected at the termination of the large-chamber incubations by clipping stems and leaves at the peat surface. Plants tissues were returned to the laboratory, dried at 110 °C overnight, and weighed.

**RESULTS**

Peat oxygen distribution. From late July to mid-October, *S. eurycarpum* plants with stem heights to 50 cm dominated the field site. Although the site was variably inundated, with water table levels ranging from approximately 5 cm above to >10 cm below the peat surface, changes in the water table were not accompanied by significant changes in the peat surface relative to a fixed horizon. Through much of August and early September, the peat surface was exposed to the atmosphere but was visibly water saturated. Water table levels rose significantly from mid-September through early winter, coincident with decreased evapotranspiration and increased precipitation. Oxygen penetration into the peat during late August–early September varied between 1 and 7 mm (Fig. 1) but was not obviously related to changes in the water table. Oxygen microprofiles under shifting light-dark regimes provided evidence for active benthic photosynthesis since oxygen concentrations and penetration depths were greater when the peat was illuminated (Fig. 1A); short-term variability in the effect of photosynthesis (compare Fig. 1A and B) may have been due in part to variable light regimes.

Methane oxidation at the peat surface. Methane oxidation was active at the peat surface, as indicated by increased methane emission in the presence of acetylene. Rates of emission from chambers containing no aboveground vegetation ranged from approximately 4 to 58 mg of CH₄ m⁻² day⁻¹ in controls to 19 to 67 mg of CH₄ m⁻² day⁻¹ after addition of 3 to 4% acetylene. Approximately 13 to 80% of the potential methane flux across the surface was oxidized. No consistent differences were observed in peat surface emission rates or the extent of oxidation from August to October. Throughout this period, emissions from the peat surface were typically 4 to 30% of the total methane emission (peat surface flux plus emission through plant leaves).

Effects of inhibitors on methane oxidation, methane production, and photosynthesis. The potential utility of acetylene and methyl fluoride in analyses of methane oxidation was examined using peat and plants (leaves and roots). Acetylene and methyl fluoride rapidly inhibited methane oxidation by washed, excised roots at concentrations from 0.01% to 1% (Fig. 2). The effect of these two inhibitors increased slightly with increasing concentration (about 86 to 89% inhibition at a concentration of 0.01% and 96 to 98% inhibition at a concentration of 1%);

although the levels of inhibition were comparable, acetylene was consistently more effective than methyl fluoride (Fig. 2).

Acetylene and methyl fluoride also inhibited methanogenesis in peat slurries incubated in vitro under anoxic conditions. Methyl fluoride inhibition was nearly total at gas phase concentrations as low as 0.01% (Fig. 3); the kinetics and reversibility of methyl fluoride inhibition were not examined. In contrast, complete inhibition of methanogenesis was not observed until addition of about 0.5% acetylene; at levels of 0.01 to 0.05% acetylene, inhibition was approximately 60% of uninhibited controls (Fig. 3).

Neither acetylene nor methyl fluoride (at 3 to 4% concentration) affected *S. eurycarpum* in vitro. Pre- and posttreatment mean photosynthetic rates ranged from 10 to 16 μmol of CO₂ (m² of leaf area)⁻¹ s⁻¹ (Fig. 4A) with no significant differences before or after gas addition (*P = 0.762, n = 15*); stomatal resistance (leaves and roots) also did not differ significantly among treatments, before or after gas addition (Fig. 4B) (means ranged from 1.5 to 2.5 mol of CO₂ (m² of leaf area)⁻¹ s⁻¹; *P = 0.139, n = 15*). Similarly, acetylene and methyl fluoride did not affect *S. eurycarpum* photosynthetic rates measured in situ during early August (Fig. 4C). Means ranged from 13.1 to 15 μmol of CO₂...
Methane accumulated linearly in chambers with *S. eurycarpum* during short-term assays after incubation with a 3 to 4% gas-phase concentration of acetylene or methyl fluoride for about 12 h (Fig. 5). Rates of methane accumulation after incubation with one of the inhibitors were consistently greater than emission rates determined during a 10- to 24-h period prior to inhibitor addition. In a comparative analysis, emission rates after incubation with acetylene or methyl fluoride were similar (Fig. 5), as was the estimated percent methane oxidation (29 and 25%, respectively); emission rates also appeared stable for up to 36 h subsequent to incubation with either inhibitor.

Methane accumulated linearly during incubation periods of 45 to 105 min in static chambers containing *S. eurycarpum*, in spite of temperature changes of up to 7°C within the chambers. Rates of methane emission were determined to be stable from morning until evening on the basis of results of time courses conducted on three separate dates; for example, rates from 8 September varied between 53.9 and 58.8 mg of CH₄ m⁻² day⁻¹ (Fig. 6). Over the period of a day, ambient air temperatures varied by about 10°C and ambient light intensities varied from <500 to >1,300 µE m⁻² s⁻¹.

Time course analyses of methane concentrations in the aboveground lacunar spaces of 20 *S. eurycarpum* leaves and *P. cordata* stems (Fig. 7) were consistent with the temporal stability of emission rates. No significant diurnal variation was observed for *S. eurycarpum*, for which leaf methane concentrations at six time points averaged 320 ppm (standard deviation, ±28 ppm). A single elevated peak at noon was not routinely observed for *P. cordata*; the mean stem methane concentration for six time points was 1.233 ppm (±193 ppm).

**Rates of methane emission and root-associated methane oxidation.** Methane emission rates varied from 53 to 178 mg m⁻² day⁻¹, with the highest rates in early August and the lowest in October (Fig. 8). Rates of methane oxidation estimated from pre- and post-acetylene treatment varied from 9.6 to 97.6 mg m⁻² day⁻¹, with peak values in mid-August. The percentage of the total methane emission that was oxidized varied from 8.8 to 43.2% based on the means for each sampling date; however, the range for all observations was from <1 to 58%, with a mean of 27.3% ± 5.5% (1 standard error). Neither pre- nor posttreatment methane emissions, methane oxidation rates, nor percent oxidation correlated with ambient temperature.

Rates of methane oxidation were not correlated with rates of pre- or posttreatment methane emission (Fig. 9A) when all
chamber observations were pooled for analysis. Likewise, percent methane oxidation was not correlated with maximum potential methane emission, although there was a weak trend for decreasing percent oxidation at the higher potential emission rates (Fig. 9B). Neither pre- nor posttreatment methane emission was correlated with the aboveground dry biomass in the chambers, which was estimated by clipping and drying all standing leaves and stems after termination of the various incubations. A trend of decreasing rates of emission with increasing biomass occurred in both cases, but neither correlation was significant ($P < 0.184$ and $0.213$, respectively).

**DISCUSSION**

In situ rhizospheric methane oxidation rates have been assayed by introducing methanotrophic inhibitors via the plant stem-rhizome-root aerenchyma system (15, 18, 19, 36). Rates estimated by this approach are based on increases in methane emission in the presence of inhibitors relative to controls. The success of this method depends on the use of inhibitors that affect methane oxidation but not methane production; alternatively, if methanogenesis is also inhibited, short-term emission rates must be controlled primarily by the supply of dissolved methane and not by instantaneous methane production.

Although acetylene inhibits methane production (31, 33, 39), data from Holtzapfel-Pschorn et al. (18) and Gilbert and Frenzel (16) suggest that short-term inhibition of methanogenesis does not affect methane emission rates in the rice rhizosphere. Nonetheless, acetylene has not been recommended for in situ assays of rhizospheric methane oxidation (15). Methyl fluoride has been proposed instead since it has been reported to inhibit methanogenesis by an estuarine methanogen and marine muds only at high concentrations (>3 to 4%). Schipper and Reddy (36) have also reported no significant methanogenic inhibition in a freshwater sediment slurry, but they apparently removed headspace methyl fluoride prior to their assays. Since methyl fluoride is a reversible methanogenic inhibitor, it is not clear that Schipper and Reddy would have observed much of an effect after removing methyl fluoride.

The effects of acetylene on methanogenesis and methane oxidation observed here are consistent with previously reported observations (see, e.g., references 21, 31, 33, and 39), as is the effect of methyl fluoride on methanotrophy (32). However, the observed inhibition of methanogenesis by low concentrations of methyl fluoride (Fig. 3) differs from results of Oremland and Culbertson (32) and Schipper and Reddy (36). Reasons for the differences may reflect (i) contrasts between marine (or estuarine) and freshwater methanogenesis, analogous to differences observed in the efficacy of of bromoethane sulfonic acid as an inhibitor (31); or (ii) the presence of methyl fluoride throughout the incubation period in this study, in contrast to the conditions used by Schipper and Reddy (36).

Regardless, the stability of methane fluxes for 24 to 36 h after the addition of acetylene or methyl fluoride to the head-
space of chambers in situ (Fig. 5) indicates that any inhibition of methanogenesis had a minimal effect on methane emission, as observed for in vitro incubations with rice (16, 18). The lack of an observable effect of methane inhibition during the chamber incubations suggests that the supply of methane for emission through plants was independent of short-term fluctuations in methanogenesis in or near the rhizosphere. While these results suggest the need for caution when using either acetylene or methyl fluoride, they also indicate that problems with short-term assays may be minimal.

In contrast to their effect on methanotrophs and methanogens, neither acetylene nor methyl fluoride affected short-term photosynthesis or stomatal resistance (Fig. 4). This is especially important since plant physiological status can affect trace gas exchange, particularly in those species for which exchange occurs primarily by active gas transport through stomates rather than by diffusive transport through micropores (24, 30). In such plants, and those with active gas transport systems (12), temperature, insolation, and relative humidity also affect trace gas exchange.

Several lines of evidence indicate that these parameters have little or no effect on methane emission from *S. eurycarpum*. For instance, the lack of diurnal variation in stem gas methane concentrations and rates of methane emission (Fig. 6 and 7) and the linearity in methane emission in spite of changes in chamber temperature agree with patterns observed for plants characterized by diffusive gas transport through micropores (7, 8, 38). Such plants are amenable to static chamber incubations for methane emission and oxidation assays as described here. In contrast, plant taxa such as the water lilies (e.g., *Nuphar* spp. and *Nymphaea* spp.) may require active control of insolation, temperature, relative humidity, and CO₂ partial pressure for similar assays.

The similarity of methane oxidation rates measured in situ with acetylene or methyl fluoride (Fig. 5), the absence of any effects on photosynthesis and stomatal resistance, and the comparable effects of both gases on in vitro methane production and oxidation indicate that either can be used for field studies of plant-associated methane oxidation. The low cost and convenience of acetylene (i.e., its availability as calcium carbide) make it a better choice than methyl fluoride for routine use. However, in contrast to acetylene, methyl fluoride is a reversible inhibitor; this may promote rapid recovery of methanotrophs after exposure to and dissipation of the gas, thereby
allowing repeated assays on the same samples (15). When such assays are desirable or necessary, methyl fluoride may prove to be the optimal choice of inhibitor. However, long-term use of methyl fluoride may require documentation that methanogenesis remains unaffected.

As has been reported for other systems (see, e.g., references 40 to 42), methane transport through plant roots and leaves dominates emission in the site described here, with methane flux across the bare peat surface contributing 4 to 30% of the total emission. Methane oxidation at the peat surface limits in part the contribution of peat fluxes; methane oxidation in the peat also appears relatively more effective than plant-associated methane consumption (14 to 80%; mean = 43%). Although there may be several controls of peat surface methane oxidation, it is likely that oxygen distribution and availability are paramount in importance. Oxygen penetration within the peat (and the distribution of active aerobic methane oxidation at the peat surface) varies from about 1 to 7 mm, with some diurnal variation coupled to benthic photosynthesis. However, oxygen penetration did not appear to fluctuate consistently with water table position in spite of excursions of up to 10 cm; this may be due to the fact that the peat remained visibly water saturated at all times. Oxygen availability has been previously proposed as an important control of methane oxidation in other wetlands (20, 23), with availability being a function of both benthic photosynthesis and heterotrophic demand as well as water table position (3, 27–29, 35).

Oxygen availability may also be a key factor limiting plant-associated methane oxidation. Although very little information exists on oxygen availability for methanotrophs in the rhizosphere of both benthic plane or rhizosphere, several observations are consistent with oxygen limitation. First, it is evident that the surface of live, healthy roots is not fully aerobic since active methanogenesis has been documented for the belowground tissues of a number of plants species (4, 22). Active methanogenesis clearly indicates that at least some fraction of live belowground biomass is unsuitable for methane oxidation. Second, methane concentrations within the root zone of aquatic plants are often >10 μM (6, 42) (concentrations typically exceed 100 μM for the site described here [5]). These concentrations are comparable to or exceed one-half saturation constants, Kₘ, measured for methane uptake by excised roots (23) and indicate that a parameter other than methane concentration or availability constrains rates. Saturating concentrations of methane would obviously imply limitation by another parameter, most likely oxygen. Third, lacunar methane concentrations measured at the stem base often exceed 1,000 ppm (equivalent to about 1.4 μM dissolved methane) and again approach Kₘ values (22, 24, 38). Since methane concentrations in the rhizosphere or rhizoplane must exceed those in the stem, limitation by a parameter other than methane is indicated. Control of root-associated methane oxidation by oxygen availability could contribute to relatively high stem methane concentrations.

The in situ emission and oxidation data also support limitations by a parameter other than methane. The postacetoxenyl methane emission rates provide a measure of total methane flux through belowground tissues and, therefore, availability of methane to root-associated methanotrophs. Postacetoxenyl methane emission rates provide a measure of the net flux after methanotrophic activity. Neither net emission nor the estimated relative extent of methane oxidation correlates significantly with the total methane flux (Fig. 9). The absence of a correlation suggests that methane availability per se was not the primary factor limiting root-associated methane oxidation during a summer-fall transition.

Parameters that could obscure a distinct relationship between methane availability and methane oxidation include temperature, plant senescence, and oxygen availability, as discussed above. Since ambient temperature generally decreased from August through October, differential responses of methane oxidation and production to temperature might contribute to an apparent uncoupling of the two processes as measured by percent methane oxidation. Changes in plant activity could have similar effects, with senescence in the fall or new growth in spring altering oxygen availability, surfaces for methanotrophic colonization, and the supply of organic matter for methanogenesis. Changes in any of these factors can contribute to short-term uncoupling of total methane flux and methane oxidation.

Schipper and Reddy (36) have also stressed the importance of oxygen for rhizospheric methanotrophy, though in a somewhat different context. They note that greenhouse studies based on the use of pot-bound plants with high concentrations of roots (i.e., high root/sediment ratios) might give misleading results because of enhanced oxygen availability relative to that in the field. Schipper and Reddy (36) also note that this phenomenon might account for the lower values of methane oxidation observed by Epp and Chanton (15) in their field incubations versus those in a greenhouse.

Arguments emphasizing the importance of oxygen availability as a control for root-associated methanotrophy in S. eurycarpum do not exclude an important role for methane availability. Methane availability may assume a predominate role in early spring with the onset of new plant growth and increased rates of methanogenesis (16). The relative importance of oxygen versus methane may also vary among plant taxa as a function of differences in oxygen transport mechanisms, the architecture of lacunar spaces, and root oxygen demand (1). For example, Gilbert and Frenzel (16) have stressed the importance of methane availability for young rice plants (Oryza sativa) on the basis of results of in vitro assays. In addition, substantial differences in root oxygenation capacity have been observed for P. cordata and S. eurycarpum, and these differences have been correlated with methane oxidation both in vitro and in situ (5). The relative importance of oxygen may also vary for a given plant species growing in habitats that differ in bulk sediment oxygen demand or nutrient supply. The former would affect competition among bacteria for oxygen, while the latter could affect the architecture of belowground plant tissues.

However, irrespective of the specific controls, methane oxidation associated with the roots of S. eurycarpum appears to consume from <1 to 58% of the total potential methane flux in situ, with a mean ± standard deviation of about 27% ± 6%. A similarly broad range, but with somewhat higher values, has been reported by others (see, e.g., references 15 and 36). Most of these studies have involved various greenhouse manipulations which, as noted above, may be unrealistic. The potential problem in extrapolating greenhouse results to field results is further illustrated by the fact that two groups who have examined methane oxidation by rice in vitro have come to different conclusions. DeBont et al. (14) have suggested that methane oxidation in the rice rhizosphere is negligible, while others (see, e.g., references 18 and 19) have reported substantial levels of oxidation.

In situ analyses of root-associated methane oxidation indicate that a significant but variable fraction of the total methane flux is consumed during the peak period of summer emission. In spite of the fact that root methanotrophy attenuates emissions significantly, it is clear that the net effect of wetland plants is to enhance methane flux to the atmosphere since emissions through plants exceed those across the peat.
surface. The large amount of belowground biosurface coupled with relatively rapid transport through lacunar gas space appears to favor methane efflux rather than consumption. The extent to which root-associated methane oxidation varies among plant taxa and among wetland ecosystems is uncertain. However, a better understanding of such variability is required since plant responses to climate change, including species succession, can profoundly affect the relative role of methanotrophs as a system level control of wetland methane emission.

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